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Poly(glycerol sebacate) — A Novel Biodegradable Elastomer for Tissue Engineering

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ABSTRACT

Biodegradable polymers have significant potential in biotechnology and bioengineering. However, for some applications, they are limited by their inferior mechanical properties and unsatisfactory compatibility with cells and tissues. A strong, biodegradable, and biocompatible elastomer could be useful for fields such as tissue engineering, drug delivery, and *in vivo* sensing [1,2]. We designed, synthesized, and characterized a tough biodegradable elastomer from biocompatible monomers. This elastomer forms a covalently crosslinked three-dimensional network of random coils with hydroxyl groups attached to its backbone. Both crosslinking and the hydrogen bonding interactions between the hydroxyl groups likely contributes to the unique properties of the elastomer. *In vitro* and *in vivo* studies show the polymer has good biocompatibility. Subcutaneous (SC) polymer implants are absorbed completely within 60 days with restoration of the implantation sites to their normal architecture.

INTRODUCTION

The current surge of research in tissue engineering (TE) underlines the urgent need for novel biomaterials designed specifically for TE. The current benchmark biodegradable polymer is polyglycolide, polylactide, and their copolymer poly(glycolide-*co*-lactide) (PLGA). These polymers were originally designed for biodegradable sutures, and have a number of drawbacks for applications in TE: (1) inferior physical property — they are rigid and brittle; (2) heterogeneous degradation — they often lose mechanical strength and crack in the early stage of degradation; (3) limited affinity for cells — they often require surface modification for wettability and cell attachment; (4) fibrous encapsulation — often an avascular fibrous capsule forms around the implant [3-6]. Here we show a novel elastomeric and strong biodegradable polymer with high affinity for cells and excellent biocompatibility.

EXPERIMENTAL DETAILS

Synthesis and characterization of the polymer. The polymer was synthesized by polycondensation of glycerol and sebacic acid. KBr pellet of newly prepared polymer was used for FTIR analysis on a Nicolet Magna-IR 550 spectrometer. DSC is measured by Perkin-Elmer DSC differential scanning calorimeter. Elemental analysis on vacuum-dried samples was performed by QTI Inc. Water-in-air contact angle was measured at room temperature using the sessile drop method and an image analysis of the drop profile with VCA2000 video contact angle system on slabs of polymer fixed on glass slides.

Mechanical properties. Tensile tests were performed on six 25x5x0.7 mm polymer strips cut from polymer sheets according to ASTM standard D 412-98a on an Instron 5542 mechanical tester equipped with a 50 N load cell. Deflection rate was kept at 50 mm/min. The samples were elongated to failure.

***In vitro* degradation.** Slabs of dry polymer (5x5x2 mm) were weighed and transferred to 15 ml centrifuge tubes filled with PBS. After 60 days, the samples were removed and washed with D.I. water. The surface water was removed by Kimwipe, and the samples were weighed after drying

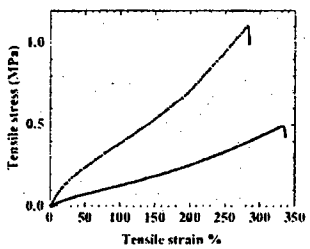

Figure 1. Stress strain curves of PGS (solid line), vulcanized rubber (dashed line). Both PGS and vulcanized rubber are marked by low modulus and large elongation ratio, indicating elastomeric and tough materials.

at 40 °C in an oven for 7 days. The degree of degradation was determined by dry weight change.

***In vitro* biocompatibility.** Nine glass petri dishes (60mm diameter) were coated with 1,3-dioxolane solution of the prepolymer (1%). The coated dishes were transferred into vacuum oven after evaporation of the solvent in air. The prepolymer was crosslinked into the elastomer after 24 hr. at 120 °C and 120 mTorr. Nine control dishes were coated with 1% CH_2Cl_2 solution of PLGA (50:50, carboxyl ended, MW 15,000), and the solvent was evaporated in 24 hr in air. The coated dishes were sterilized by UV radiation for 15 min. Each dish was soaked in growth media for 4 h, replaced with fresh media and soaked for 4 h before cell seeding to remove any unreacted monomers or residual solvents. Each dish was seeded with 100,000 NIH 3T3 fibroblast cells and 8 ml of growth medium. The cells were incubated at 37 °C with 5% CO_2. Cell density was measured by MTT assay [7]. Media exchange was performed every 48 h. At day 6, phase contrast images were taken for both the polymer wells and the control wells on a Ziess Axiovert 200 microscope equipped with a Dage 240 digital camera.

***In vivo* biocompatibility.** Autoclaved PGS slabs of approximately 6x6x3 mm, and ethylene oxide sterilized PLGA disk (2 mm thick, 12.5 mm diameter) were implanted SC. in 15 seven-week-old female Sprague-Dawley rats (Charles River Laboratories) by blunt dissection under deep isofluorane/O_2 general anesthesia. The surface area/volume ratio were kept the same for both PGS and PLGA implants. Two implants each of PGS and PLGA were implanted symmetrically on the upper and lower back of the same animal. Every implantation site was marked by 2 tattoo marks 2 cm away from the implantation center. The animals were randomly divided into 5 groups. At each predetermined time point, one group of rats was sacrificed, and

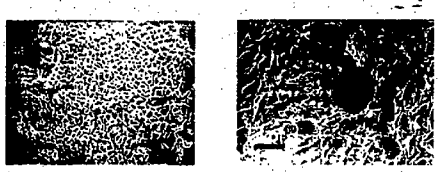

Figure 2. Comparison of NIH 3T3 fibroblast cell morphology and number in PGS sample wells (left) and PLGA control wells (right) 6 days after seeding. The PGS wells had more adherent cells and the cell morphology appeared normal, while those in the control well adopted a long thin thread like shape. Scale bar = 200 μm.

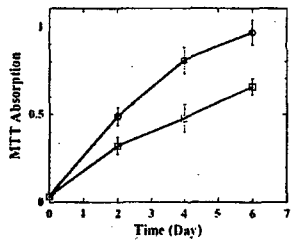

Figure 3. Comparison of growth rate of NIH 3T3 fibroblast cells in PGS ( ) wells and PLGA ( ) wells. MTT absorption measured at 570 nm, normalized value shown.

tissue samples (~15x15 mm) surrounding the implants were harvested with the intact implant. The samples were prepared by standard methods.

RESULTS AND DISCUSSION

PGS features ester cross-links and hydroxyl groups directly attached to the backbone [8]. The C=O stretch at 1740 cm^{-1} in Fourier transformed infrared (FTIR) spectrum confirms the formation of ester bonds. FTIR also shows an OH stretch at 3448 cm^{-1}, which reflects the presence of hydrogen bonded hydroxyl groups in the molecule. Elemental analysis confirms the composition of PGS as approximately 1 glycerol:1 sebacic acid. PGS is very hydrophilic due to the hydroxyl groups attached to its backbone. The water contact angle of PGS is 32°. Since PGS is hydrophilic by nature, unlike PLGA, surface modification is unnecessary to make it wettable.

Appropriate cross-link density renders PGS elastomeric and tough. Tensile tests on PGS strips reveal a stress-strain curve characteristic of a soft and strong material (Fig. 1). The shape of the stress-strain curve is similar to that of vulcanized rubber [9] and tendon [10]. In the elongation test, the samples can be elongated repeatedly up to at least 300% of its original length without rupture. The total elongation is unknown, as grip breaks occurred at about 267±59.4% strain. Compression tests indicate that the material can be compressed up to 70% repeatedly without rupture.

PGS also appears to be biocompatible both *in vitro* and *in vivo*. NIH 3T3 fibroblast cells were seeded homogeneously on PGS coated glass petri dishes with PLGA coated dishes as controls. The cells in PGS sample wells are viable and showed normal morphology with higher growth rate than the control, as tested by MTT assay [11] (Fig. 2A, Fig. 3). Cells in PLGA wells tend to form clusters, and the number of floating cells are higher, furthermore, most of the attached cells adopted a long thin thread-like morphology (Fig. 2B). These experiments suggested that PGS is at least as biocompatible as PLGA *in vitro*.

SC implantation in Sprague-Dawley rats was used to compare the *in vivo* biocompatibility of PGS and PLGA. These PGS and PLGA implants have the same surface area/volume ratio (1.33±0.04). Both PGS and PLGA samples were implanted symmetrically on the back of the same animal. The inflammatory responses subsided with time for both polymer implants. In the first three weeks, the inflammatory response of PLGA implantation sites were about 16 % thinner than that of PGS (Fig. 4). The thickness of the inflammatory zone in both implantation sties were approximately the same at week 4 and 5. Fibrous capsules surrounding PLGA

implants developed within 14 days, and their thickness hovered around 140 μm. Collagen deposition did not appear around PGS implants until 35 days. The collagen layer was highly vascularized and was only about 45 μm thick. The inflammatory response and fibrous capsule

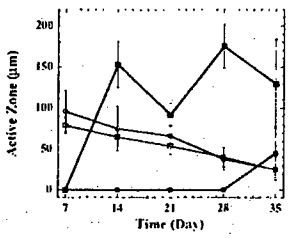

Figure 4. Change of thickness of the immune responses with time for PGS and PLGA. The inflammatory response decreased with time for both polymers, which had similar inflammatory zone thickness. While the thickness of fibrous capsules surrounding PLGA was consistently and significantly larger than that of PGS. Inflammatory zone: PGS, ○, PLGA, □; fibrous capsule: PGS, ●, PLGA, ■.

formation observed for PLGA is similar to those reported in the literature[12,13]. Thick fibrous capsules block mass transfer between the implants and surrounding tissues, which can impair implant functions. In an *in vivo* study with PGS alone, the SC implantation sites were undetectable despite repeated sectioning of the specimens at multiple levels in 60 days (2 implantation sites each in 3 animals). The implants were completely absorbed without granulation or scar tissues, and the implantation site was restored to its normal histological architecture. Overall, the inflammatory response of PGS is similar to that of PLGA. However, unlike PLGA, PGS barely induces any significant fibrous capsule formation.

The degradation characteristics of PGS are examined both *in vitro* and *in vivo*. Agitation for 60 days in phosphate buffered saline solution (PBS) at 37 °C causes PGS to degrade 23%. In fetal bovine serum (FBS), the degradation is 39% under the same conditions. The degradation rate is the slowest for PBS, faster for FBS, and fastest *in vivo*. This suggests that enzyme degradation occurred in the latter two cases, and the action of macrophages in the body causes PGS to degrade even faster. The PGS explants maintain their square shape and sharp edges up to at least 31 days. Furthermore, preliminary test of the explants with a nano-indenter indicates that the decrease of mechanical strength parallels that of the mass. Both suggest PGS most likely undergoes surface erosion.

CONCLUSIONS

Compared with existing biodegradable elastomers, PGS appears to be tougher, inexpensive, and more flexible. In the models tested, the material is biocompatible both *in vitro* and *in vivo*. The polymer's properties, such as hydrophilicity, degradation rate and pattern can potentially be tailored by grafting hydrophobic moieties to the hydroxyl groups[14,15]. To further control or regulate polymer interaction with cells, biomolecules could be coupled to the hydroxyl groups or integrated into the polymer backbone[16-18].

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